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Introduction

The fact that the horse is seasonally polyestrous makes this species a reproductive challenge for horsemen and equine practitioners. Mares will show several estrous cycles during the breeding season assuming pregnancy does not terminate estrus. The receptive season is limited to such a length of time that parturition occurs in the spring of the year. The challenge arises when trying to devise methods that would allow parturition to take place earlier in the year. This is desirable only because most breed registries have imposed an arbitrary birthdate of January lst to all foals born in a single year. Each yearling is considered one year of age on January 1 of each year regardless of their actual age. Concern among horseowners regarding this stipulation lies in the eligibility of their horses for age limited races, shows, or events. In order to be competitive, actual birthdates as close to January 1 are desired.

The gestation length of the mare is approximately 330-340 days. Therefore, breeding dates in February, March, and early April with the resulting January, February, and March foalings, offer the greatest competitive edge. However, in the Northern Hemisphere, 75 to 80% of the mares are in deep winter anestrous or early transition at this time.[6]

In a seven year study, it was found that the mean date of first ovulation for Thoroughbred and Quarterhorse mares was April 1st.[14] June and July are the months where peak sexual behavior can naturally be observed, while November usually marks the beginning of winter anestrous.

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Neural and Endocrine Control of the Estrous Cycle

The main contributing factor in the seasonal breeding pattern of the horse appears to be directly correlated to photoperiod. During the spring and summer months, with increasing daylight periods peaking at 15 to 16 hours, ovarian activity is stimulated while the shorter daylight (9 to 10 hours) during winter months inhibits ovarian activity.[6] Researchers have found that exposure of the retina to light sends a neural stimulus through the optic nerve to the pineal gland in the brain.[5] It is then thought that the pineal gland functions as a neuroendocrine transducer converting neural input to hormonal output. The major hormone produced by the pineal gland affecting reproduction seems to be melatonin. It has been shown that a depletion in gonadotropin releasing hormone (GnRH) resulted in ovariectomized mares after the insertion of melatonin implants.[15] The synthesis of melatonin is inversely related to the duration of retinal light exposure. Increasing light causes a decrease in melatonin production while decreasing light results in increased production.[6] Therefore, the activity of the ovaries and the seasonal breeding pattern of horses can be directly related to the duration of light exposure, melatonin synthesis, and ultimately GnRH production.

GnRH is a decapeptide produced by the hypothalamus.1 Through a system of portal vessels, GnRH is transported from the hypothalamus to the adenohypophysis (anterior pituitary) where it assists in controlling the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[5] It is thought that the frequency of pulsatile release of GnRH determines the rate of release of either FSH or LH. High frequency pulses cause release of LH while low rates result in FSH release.[10]

Follicular development, ovulation, and corpus luteum (CL) formation by the ovaries are all controlled by FSH and LH. It is recognized that a bimodal release of FSH occurs with two peaks per estrous cycle at 10 to 11 day intervals. Two waves of follicular growth coincide with the two FSH peaks.[6] Those follicles that result from the FSH stimulation at days 9 to 12 reach maturation by the end of estrus.[5] LH is the gonadotropin responsible for ovulation. LH levels begin to rise at the beginning of estrus.[6] A selection mechanism, theorized to be follicular LH receptor sites, designates one, sometimes two, and rarely three or more follicles to undergo final maturation and ovulation. Peak LH occurs approximately two days post ovulation and returns to baseline concentrations by days five or six and remains low until the next estrus.[5]

The prolonged rise in LH, from the beginning of estrus to two days post ovulation, is necessary for the stimulation of follicular granulosa cells to transform into luteal cells. The luteal structure that first develops is termed the corpus hemorrhagicum which matures to become a CL. This structure begins to produce progesterone by day one or two after ovulation. The progesterone production by the CL continues to rise until around day 6, when concentrations then plateau. The mature CL will produce progesterone at levels equalling 8 to 10 ng/ml until approximately day 14 or 15 of the estrous cycle. Luteolysis of the CL occurs at this time, in response to prostaglandin F2 (PGF2a) being released from the endometrium of the uterus. Progesterone concentrations rapidly fall to levels less than 1 ng/ml within one to two days, and the mare returns to estrus.[5] Progesterone functions in coordinating the estrous cycle through negative feedback effects on LH secretion and acts as the "hormone of pregnancy". It blocks estrus behavior, suppresses myometrial activity, promotes endometrial secretion, and acts synergistically with estrogens to promote uterine and mammary growth.[11] Therapeutic usage of progesterone will be presented under the section discussing the transitional phase.

Artificial Light

It is most likely that the onset of winter anestrous occurs due to insufficient levels of LH. It has been shown that mares usually enter winter anestrous due to failure of ovulation following normal regression of a CL. This is the result of the shortening daylight period which stimulates melatonin production resulting in decreased GnRH release.

Better understanding of the reproductive pattern of mares and its relationship to photoperiods has led to the development of exogenous means to control it. It has become a popular practice of breeding farms to start artificial light supplementation in late November or early December to achieve a breeding season starting in February. The method most often used is to extend light exposure past sunset by artificial means to equal 16 total hours per day.[6] The light intensity requirement is 10-12 foot candles. This can be provided by one 200-watt incandescent light bulb per 12 by 12 foot box stall.[15]

A simple method of testing the intensity of light in a stable is by use of a 35mm camera with a through-the lens light meter. With the film speed setting at ASA 400 and the shutter speed setting at 0.25 seconds, a clean, plain white styrofoam cup is placed over the lens. The cup acts as a diffuser and averages the light from all the point sources in the stable. The aperture setting is read with the camera held at about the height of a horses' eye. Readings of greater than f4 indicates an adequate light intensity.[15]

An alternative to stall lighting is to use lighted paddocks. Several mares can be placed in properly lighted paddocks at the end of the day. The lights can be controlled by timers and automatically turned off after the required 16 hours of total light has been met. Automatic gate openers can then be set to allow the mares to return to pasture.[15]

Transitional Phase

The use of progesterone or synthetic progestogens have been used to try and shorten the anestrous period.[12] However, this treatment is only efficacious when used on mares that have already advanced into the transitional phase of the estrous cycle. This period occurs between deep winter anestrous and the onset of normal estrous cycles. During this time, estrous behavior is irregular and inconsistent. The ovaries are larger than during winter anestrous and begin developing many 10-20 mm follicles. The number of follicles remain high until one is selected to become the preovulatory follicle. Prolonged estrus behavior can last from one week to four months or more.[6]

Some of the original work in the area of progesterone treatments for mares in transition, showed that 100 mg of progesterone in oil, administered once daily for seven days, terminated the prolonged estrus in approximately two days and decreased the abundant follicular activity. Following the last injection the mares returned to estrus within three days and normal cyclicity continued.[5] Since then an oral progestogen (altrenogest, Regu-mateR, Hoechst Roussel) has been developed that has shown efficacy in the treatment of transitional mares. The recommended dosage for this product is 1 ml per 110 pounds of body weight (0.044 mg/kg), given once daily for 15 consecutive days. Return to estrus has been shown to occur within three days post treatment and normal cyclic activity follows.[11]

A third method consists of a combination of progesterone and estradiol-17B. Ten daily intramuscular injections of 150 mg of progesterone plus 10 mg of estradiol-17B has shown good success. While estrogen alone stimulates LH release, when combined with progesterone it causes suppression of LH to near nondetectable levels.

It is important to the success of these treatments that, prior to their initiation, the mare must have very active ovaries containing several follicles that are at least 15 to 25 mm in diameter. The mechanism of action making progesterone or progesterone plus estradiol-17B, effective for treatment of transitional mares is blockage of LH release. What is most likely occurring in these mares is insufficient production of LH. These LH levels are not capable of causing final follicular maturation and ovulation to take place. While being suppressed in the presence of progesterone,LH levels surge after treatment is stopped. The mare returns to estrus, and normal follicular maturation, ovulation and CL formation results.

Research is currently underway to develop a better method of progesterone administration. Intramuscular injections, as well as, oral dosing is labor intensive, and requires adequate facilities. Therefore, creation of prolonged release devises, such as, impregnated vaginal sponges or subcutaneously injectable implants would be very beneficial.

Induction of Ovulation During Anestrus

Pituitary Extract

The first report of successful induction of ovulation in mares during winter anestrous by means of exogenous hormones was accomplished by Wisconsin workers. In this same study they were also the first to achieve induction of multiple ovulation in mares. Their experiment was conducted to test the hypothesis that ovulation could be induced in winter anestrous mares with equine pituitary extracts. Mares chosen for the study were only those with small, inactive ovaries containing follicles less than 10 mm in diameter. Also, venous blood was collected one week prior to the start of the experiment for progesterone quantitations. Only mares with a peripheral plasma progesterone concentration of less than 1 ng/ml were included in the experiment. This criteria proved that the mares were truly anestrus with no ovarian activity present.

The experiment resulted in 87% of all treated mares ovulating following 14 days of twice daily injections. Of these mares, 58% had two or more ovulations per estrus. The days to first ovulation averaged 14 to 15 days from the start of treatment. No ovulations occurred in controlled mares during the time the experiment was conducted.[3]

GnRH and Progesterone

Since the experiment conducted by the Wisconsin workers, attempts to induce ovulation and multipleovulations in winter anestrous mares has involved treatments with GnRH. Some of the original work was conducted by Evans and Irvine. It was their intention to stimulate, by exogenous administration of GnRH and progesterone, the events that take place in the normal ovulatory cycle. Courses of GnRH were administered at approximately ten day intervals. Progesterone was administered from days six to 23 in attempt to reproduce the luteal phase. In the experiment, 13 deeply acyclic mares treated with this regimen developed follicles ranging in size from 20-70mm, that subsequently ovulated. Only one of five untreated.mares showed any ovarian development during this same time period.[4]

Pulsatile GnRH Administration

Since the work conducted by Evans and Irvine, A. L. Johnson et al. has conducted a study evaluating the effectiveness of pulsatile GnRH administration to induce multiple ovulations in anestrous mares. The experiment was conducted in mid January to the first week in February. Mares were randomly allotted to one of four treatment groups. A control group was used along with one group receiving 2 ug GnRh/ hour, another receiving 20 ug GnRH/hour and the fourth 100 ug GnRH/hour. Administration was via an indwelling infusioncatheter. The GnRH was given as a single five-second pulse in 118 ul saline. The results of the experiment showed failure of all control mares to ovulate or show any significant follicular development. The number of days to first ovulation was comparable in all three treated groups; however, the number of follicles ovulating was significantly higher in groups receiving 20 ug GnRH/hour and 100 ug GnRH/hour. Double ovulations occurred in three of nine mares in the 2 ug GnRH/hour group. The normal incidence of double ovulations in the equine is estimated to range from four to 43%. In consideration of this fact these researchers suggest that the 2 ug GnRH/hour group most closely resemble a normal ovulatory response.[9]

GnRH Implants

Most recently, Australian researchers (J. H. Hyland et al.) have developed a means to administer a constant infusion of GnRH. Continuous 28 day release of GnRH is achieved by surgically implanting an osmotic minipump under the skin of the neck.[8] Various pump dosages ranging from 100 ng/kg/28 days to 400 ng/kg/28 days are used depending on the size of the mare. Ovulation occurs on the average in 20 days (range:5-32 days) following pump insertion. Normal cyclic activity is then observed post ovulation with the pump in place and after it's removal. These individuals are now in the process of conducting field trails in the United States, using privately owned mares.

While the osmotic minipump being used by the Australian group has shown that constant infusion of GnRH via an implant will induce ovulation in acyclic mares, it is still a less than adequate device for commercial usage. R. H. Douglas of BET Laboratories is in the process of developing a more practical implant. By the process of microencapsulation, he feels an implant can be made that would allow it to be injected subcutaneously and remain impalpable through the skin. This type of implant dissolves slowly releasing daily levels of GnRH sufficient to induce ovulation. Eventually the implant is completely dissolved eliminating the need for removal. In an experiment conducted by W. R. Allen, testing the effectiveness of a GnRH analogue for inducingovulation in acyclic mares, a similar implant as described above was used. It was injected under the skin via a 14 gauge needle and was not palpable after its insertion. Results comparable to those recorded by Hyland were achieved in this study.[1]

Gonadotropins

Direct stimulation of ovarian activity in anestrous mares by exogenous gonadotropins (rather than indirectly, by use of GnRH), may be the eventual treatment for this condition. The genetic code of equine FSH and LH has been mapped. Large quantities could be produced by recombinant DNA techniques if production of these hormones were warranted by industrial demand. Research protocols involving gonadotropin regimens will most likely soon result. It may be found that mimicking the normal estrous cycle exogenously through gonadotropin supplementation can stimulate ovulation in acyclic mares as well. Then more exact daily hormone levels could be determined in order to achieve minimal requirements. Perhaps an FSH implant followed by an injection of LH on a determined day will be the treatment of the future.

Added benefits of having equine gonadotropins would occur in the area of superovulation research. In the past equine pituitary extract has been most effective in stimulating multiple ovulations. Equine pituitary extract, however, is quite expensive to produce, limiting its use in this area.

Also equine LH would be superior over the currently used Human chorionic gonadotropin (hCG). hCG is widely used in the equine breeding industry to induce ovulation in cyclic mares. hCG can be given at dosages of 2000-3000 IU intravenously during early estrus when there is one large dominant follicle. It has been shown, in order to be effective, the follicle should be equal to, or greater than, 35 mm in diameter.[7] Mares responding to hCG usually ovulate within 48 hours following the injection. With the ability to control ovulation, avoidance of stallion overuse as well as, minimizing uterine contamination by penile microorganism, particulary in subfertilemares, can be achieved. Mares may produce antibodies to the globulin component of hCG limiting its use for multiple treatments. Subsequently equine LH would be superior.

Conclusion

At the present time the method that is most feasible and practical for shortening the anestrous period is an artificial lighting program. Added to this, development of a good teasing program starting in early February is crucial. Examination of all mares not showing normal cyclicity should then be done. Rectal palpation and ultrasound of the ovaries and reproductive tract are important diagnostic aids, as are vaginoscopic exams and plasma progesterone levels. Those mares presenting with prolonged, abnormal estrus with active ovaries, relaxed, pale, dry cervix and plasma progesterone less than 1.0 ng/ml should be considered to be in the transition phase. Those failing to show estrus and having active ovaries, tight, pale, dry cervix and plasma progesterone greater than 1.0 ng/ml should be considered to contain a persistent CL and can be successfully treated with prostaglandins.[11]

Alternatives to the above approach such as the use of exogenous GnRH or gonadotropins offers exciting possibilities. However, as we wait for further development in these techniques, proven treatments and patience are best practiced.

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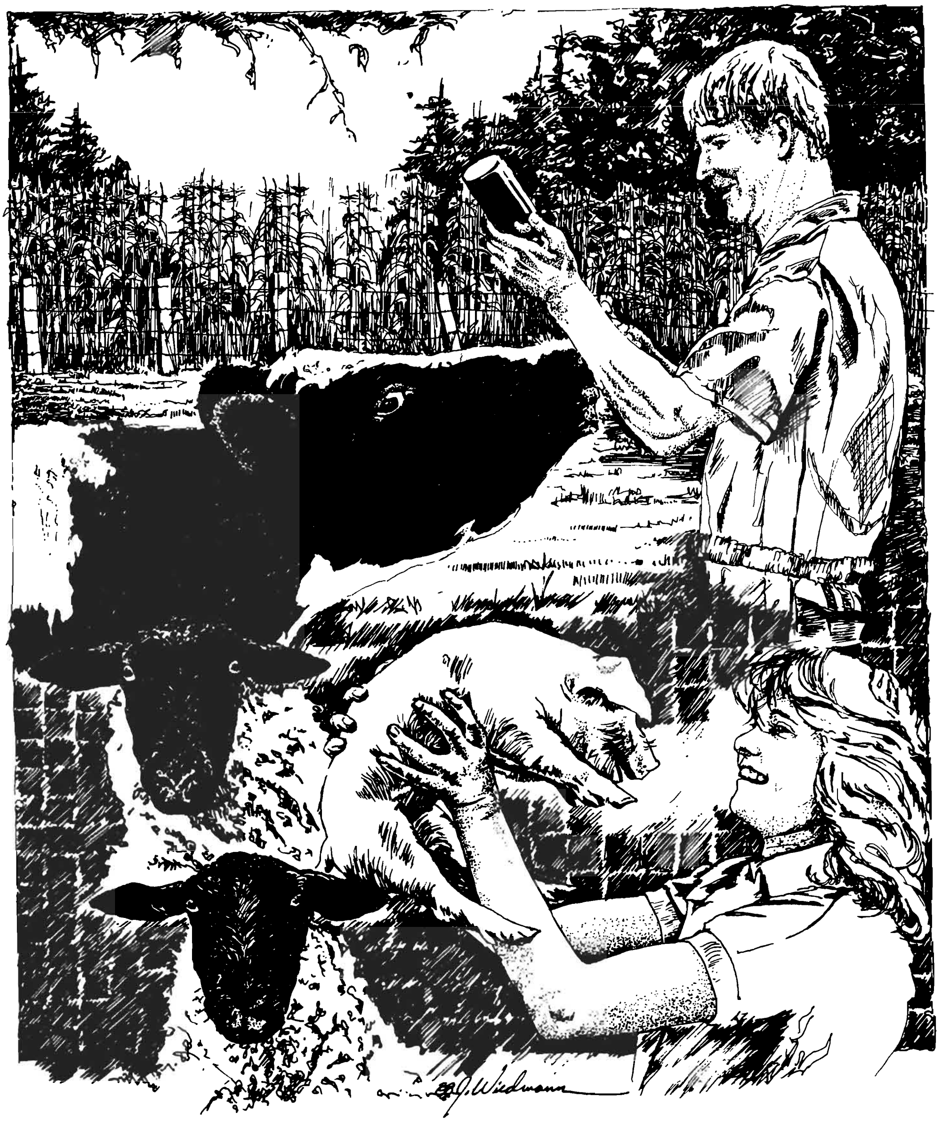

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